

## **Referring to the Review 1.**

Thank you very much for the in-depth review of our manuscript. We are very grateful for the appreciation of our laborious study on the ultrastructure of hepatocyte mitochondria in pediatric nonalcoholic steatohepatitis.

As for the question concerning differential diagnostics (whether hemochromatosis was included) we would like to explain that we did not perform genetic research in this field. However, we should emphasize that our ultrastructural assessment excluded hemochromatosis. We did not observe accumulation of iron deposits within hepatocyte cytoplasm or Kupffer cells - there were no granular electron dense iron particles in the secondary lysosomes and free in the hyaloplasm.

The final diagnosis of pediatric NASH was based on the morphological assessment of liver biopsies, i.e. on the analysis conducted with a light microscope and using electron-microscopic investigations.

Referring to nuclear morphology, since the subject of our manuscript was ultrastructural analysis of hepatocyte mitochondria, we did not focus on the submicroscopic of cell nuclei. However, we are grateful for the suggestion of such investigations and for the latest interesting work on the animal model of NASH - a mouse model of diet-induced obesity and hepatic steatosis, conditions which can be associated with hepatic neoplasia – eg: Iatropoulos MJ, Duan JD, Jeffrey AM, Leach MW, Hades AN, Stedman NL, Williams GM. Hepatocellular proliferation and hepatocarcinogen bioactivation in mice with diet-induced fatty liver and obesity. *Exp Toxicol Pathol.* 2013,65, 451-6.

The above publication will be an important inspiration for the extension of our study with ultrastructural analysis of hepatocyte cell nuclei in children with this pathology.

## **Referring to the Review 2.**

Thank you for the time and effort that the Reviewer devoted to our manuscript.

Ad. 1. We would like to explain that we suggest that electron microscopic examination is useful as a diagnostic method in the diagnosis of NASH in pediatric patients, since in 9 out of 10 cases of this pathology we found repetitive characteristic ultrastructural changes in hepatic mitochondria. These were mainly megamitochondria with crystalline inclusions (MMC). The mitochondrial abnormalities were very similar to those observed by Caldwell SH, Swerdlow RH et al. (*J. Hepatol* 1999; 31: 430-434); Le TH, Caldwell SH et al. (*Hepatology* 2004; 39: 1423-1429) in adult patients with NASH.

Ad. 2. We did not perform genetic investigations for hemochromatosis and citrin deficiency in NASH children.

However, we should emphasize that our ultrastructural assessment of liver biopates excluded hemochromatosis. We did not observe accumulation of iron deposits within hepatocyte cytoplasm or Kupffer cells - there were no granular electron dense iron particles in the secondary lysosomes (hemosiderosomes) and free in the hyaloplasm.

We diagnosed NASH based on the morphological assessment of liver biopates from the study children, i.e. on the analysis conducted with a light microscope and using electron-microscopic investigations.

Referring to the suggestion that Discussion should include more details about etiology and mitochondrial morphologic changes, we would like to add that in our comments on the ultrastructural changes in these organelles we included chosen liver pathologies, i.e. early phase of alcoholic steatohepatitis, copper metabolism disorder, i.e. Wilson's disease and NASH in adult patients, as well as rodent nutritional model of non-alcoholic steatohepatitis. Obviously, the discussion could be extended by mitochondrial abnormalities in other pathologies. This, however, would by far increase the volume of the manuscript that is intended to be Brief Article.

### **Referring to the Review 3.**

We are very grateful to the Reviewer for the time and effort devoted to our manuscript and for an interesting suggestion added to the review.

**Ad. 1.** The presented electronograms were obtained from various pediatric patients with nonalcoholic steatohepatitis. According to the suggestion, we added this information in the description of figures.

**Ad. 2.** We did not perform that sort of assessment in our NASH children (ultrastructural quantitation referring to hepatic stellate cells, e.g. the volume occupied by these cells in the space of Disse). We rather focused on the electron-microscopic analysis of hepatic mitochondria in the course of NASH.

Thank you very much for the suggestion of such observations. We will use it in our further morphological studies on pediatric NASH.